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Can overcrowding a BSC work area lead to **a loss of containment?**

WITH

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ABSTRACT

Work conducted in Biosafety Cabinets (BSCs) is a common sight in many laboratories worldwide, but some misconceptions about their capabilities sometimes arise. A BSC used for storage of materials or the air grilles or grates blocked is often observed, but no conclusive evidence has shown whether this will affect the BSCs performance.

Here we explored and tested the effect of overcrowding a cabinet as well as blocking off the front grille to determine if it will maintain ISO Class 5 air cleanliness.

Not surprisingly, once the airflow patterns within the cabinet were compromised, containment was drastically decreased, increasing the risk of contamination occurring to either the work or the worker. Therefore, storing materials within a BSC and especially blocking the front intake grille is highly discouraged.

INTRODUCTION

Biosafety cabinets (BSCs) are a common tool used in many laboratories to provide contamination control for the experiments and a safe working conditions for the workers. These BSCs depend on directional airflow and HEPA filters to remove contaminants from the environment and prevent their spread, requiring proper usage of the BSC. It is common to see BSC users blocking the front intake grille or rear suction slots (**Figure 1**) with various materials such as absorbent pads ("diapers") or lab notebooks while working within the BSC as well as filling the work area with various supplies and using the cabinet for storage instead of just work (**Figure 2**). In the BSC manuals, it states that these cabinets should be operated with the vents free from obstruction and not used to store any materials, but that is not always translated into practice.

The impacts on airflow dynamics within the BSC with front intake grille blockage and overcrowded work areas are little known to researchers and laboratory personnel as both directly correlate to the BSC's ability to provide safe and clean working conditions for both personnel and products involved (**Figure 1**). It is important to understand how airflow functions in a hood and what could be potential factors in affecting its proper flow and function. Under non-cluttered working conditions without the front intake grille covered, the air travels into the front intake grille to prevent any contaminates from escaping. The air will then travel up through the back and side plenums to the motor/blower. Here the air splits, and a portion of the air will be pushed through the supply HEPA filter and unidirectional downflow air will flow down to the work surface where it provides product protection to the work being done. The air will then split, and some will return through the back return slots, and some will flow into the front grille.

The remainder of the air will be exhausted through the exhaust HEPA filter to provide environmental protection to the surrounding areas. According to NSF International Standard 49, the criteria for build and functioning operations of BSCs, the cabinet should maintain a minimum standard of air cleanliness (NSF, 2018).

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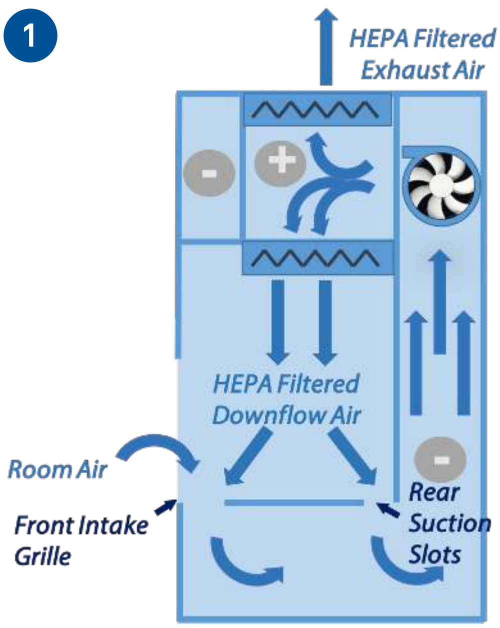

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__Figure 1__

Class II Type A2 Biosafety Cabinet schematic depicting airflow patterns and design features.

__Figure 2__

Experimental set up showing 25% of the work surface covered, 25% of the front intake grille blocked, and the particle counter and probe set in place within the BSC.

Using ISO 14664-1, these BSCs should maintain at least ISO Class 5 air when tested in a Class 8 environment (**Figure 3**, ISO, 2015).

Here we tested the ability of the 4-foot BSC to maintain ISO Class 5 air cleanliness standard with a proportion of the front intake grille blocked moving from left to right, as well as overstocking the BSC work area at increments of 25%, 50%, 75%, and 100% surface area coverage.

This testing was to simulate over usage of the cabinet, as well as placing items on the front intake grille such as a notebook, worksheet, or absorbent pad and determine if the BSC can maintain a safe environment for the worker and the work being conducted.

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ISO 14644-1 Cleanroom Standards

Maximum particles /m³						
Class	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥5 µm
ISO 1	10	2.37	1.02	0.35	0.083	0.0029
ISO 2	100	23.7	10.2	3.5	0.83	0.029
ISO 3	1,000	237	102	35	8.3	0.29
ISO 4	10,000	2,370	1,020	352	83	2.9
ISO 5	100,000	23,700	10,200	3,520	832	29
ISO 6	$1x10^6$	237,000	102,000	35,200	8,320	293
ISO 7	$1x10^7$	$2.37x10^6$	1,020,000	352,000	83,200	2,930
ISO 8	$1x10^8$	$2.37x10^7$	$1.02x10^7$	3,520,000	832,000	29,300
ISO 9	$1x10^9$	$2.37x10^8$	$1.02x10^8$	$3.52x10^7$	8,320,000	293,000

Figure 3

ISO 14664-1 Air Cleanliness Standard. Maximum particles allowed for each classification measured at different particle sizes.

METHODS

All testing and procedures were carried out in a 4 foot Class II Type A2 BSC (SterilGARD SG404, The Baker Company) that has been balanced at factory determined setpoint with an 8 inch sash opening. A background challenge of approximately 100,000 particles per cubic foot (3,520,000 particles per cubic meter) was achieved with an aerosol generator using PAO according to the ISO 14644-1 standard for Class 8 specifications using 0.5 µm particles and measured prior to running each test using a MetOne Model A-2408 Laser Particle counter.

The sampling probe of the particle counter was set at a plane 4 inches below the bottom of the sash within the work area, and readings were taken left to right at approximately 4.5 inches intervals along the front grille from left to right.

The particle counter was set to test for 0.5 µm sized particles and take readings at 1.56 cubic meters/minute. The air cleanliness testing involved two main parameters: % of surface area covered with lab materials and % of front grille blockage using an absorbent pad or "diaper". The front grille and cabinet overloading were measured in quartiles for simplicity.

All particle counts acquired during testing was converted as described in the ISO 14644-1 air cleanliness standard. A result was determined as "passing" if it was below the limitation for ISO class 5 particles. If the particle count was greater, it was deemed to "fail".

Data manipulation and statistical analysis was conducted in Microsoft Excel and GraphPad Prism ver 5.00.

RESULTS

Covering the work area obstructs airflow throughout the BSC, leading to breaches of containment and potential contaminations. As seen in Figure 4, there is a direct inverse correlation between work area coverage and the ability of the BSC to maintain ISO Class 5 air. Interestingly, when increased portions of the front intake grille were covered, the ability of the BSC to maintain ISO Class 5 air was decreased (**Figures 4 and 5,** r2 = 0.9967). In Figure 4, a leftward shift of the lines indicate that ISO Class 5 air was not achieved with less coverage of the work area if the front grille was covered.

Covering of the front intake grille of the BSC was directly correlated to the BSC failing to reach ISO air cleanliness standards (**Figure 4**). When 100% of the front grille was covered, nearly all the tests also failed. The amount of coverage of the work area negatively trended with ability to meet ISO Class 5 air within the BSC. The least amount of success to reach ISO Class 5 air was seen when 75% and 100% of the surface area was covered (**Figure 4**), however, if the front access opening grille was completely uncovered, the cabinet was sometimes able to maintain ISO Class 5 air. However, this was not consistent, and may still pose a significant risk to the worker or work being conducted within the cabinet.

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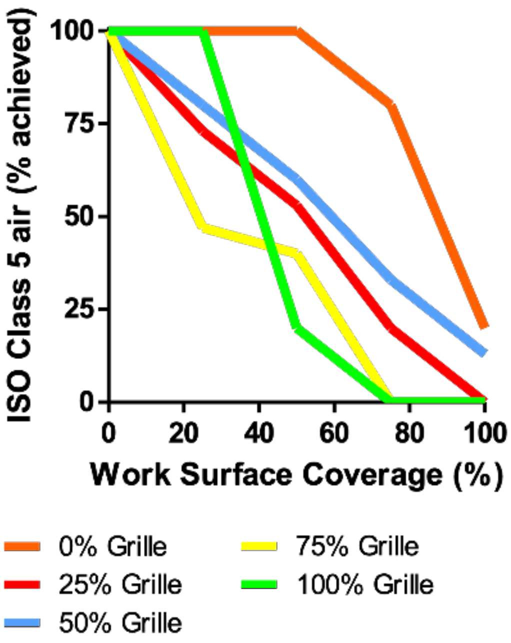

Figure 4

Correlation between Work Surface and Front Intake Grille coverage and the ability of the BSC to maintain ISO Class 5 air.

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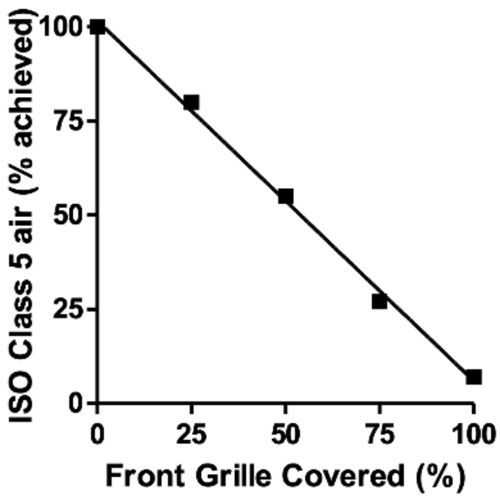

Figure 5

The correlation between coverage of the front intake grille and ability of the BSC to maintain ISO Class 5 air is shown. r2 = 0.9967

Certain regions of the BSC were determined to have the highest level of particle escape leading to failure to meet ISO Class 5 specifications, specifically the left half of the cabinet just inside the front access opening (0-36", **Figure 6**). Once the front grille was covered in these "hotspot" locations, it was common to see increases in the particle counter readings, meaning more particles or potential contaminants were able to enter the BSC.

We finalized our testing procedures with setting up a scenario that might mimic an exaggerated overcrowded BSC. We filled the work area with lab items and materials, covering the entire surface area for the hood except for a 6-inch zone at the front center of the cabinet (**Figure 7**). These tests yielded consistent failure to reach ISO Class 5 air cleanliness standards, except for when the front grille was not covered at all (**Figure 8**).

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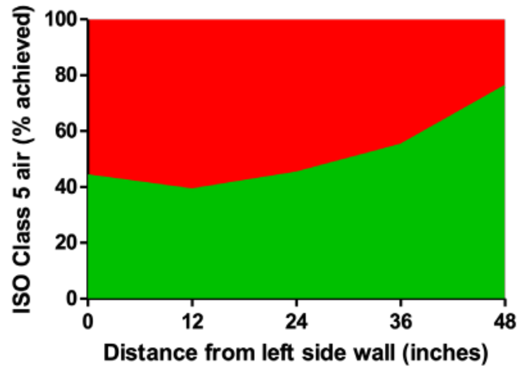

Figure 6

Locations within the BSC along the front access opening vary slightly in the ability to maintain ISO Class 5 air.

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Figure 7

An exaggerated work set up with the entire work area of the BSC covered excepting a small 6-inch region in the center and 50% of the front intake grille covered, with the particle counter probe in place.

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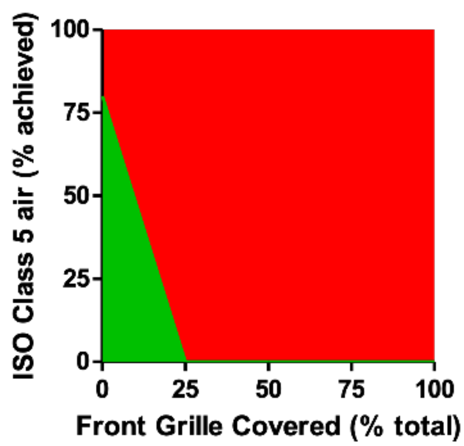

Figure 8

Percentage of Failed tests as the front grille is covered when the work surface is completely full excepting a 6 inch work area.

CONCLUSIONS

These experiments show a strong correlation between covering the work area and the inability of the BSC to maintain the required ISO Class 5 air cleanliness, which will increase the risk of contamination. Additionally, when the front intake grille is similarly covered, the BSC it is even less likely that the BSC will reach ISO Class 5 air within the cabinet (**Figure 4**). Covering the front intake grille is commonly seen with notebooks which are equal to roughly 25% of the grille, or absorbent pads or "diapers" (50% coverage each) to catch any spills that may occur during work conducted in the BSC.

The exaggerated scenario tested where the entire cabinet was filled except for a small 6-inch area showed how problems can quickly escalate (**Figure 7 and 8**). This may seem extreme, but this situation has been witnessed in laboratories across the world.

While the ability of the BSC to maintain ISO Class 5 air cleanliness conditions may be a decent percentage, it is not always 100% when there is some occlusion of the airflow. This has the potential of translating into serious contamination risks to either the work, experiment or the user. Depending on the biohazard, this is not a risk worth taking. Therefore, it is strongly recommended that all air vents within the BSC both front and back are kept free of blockage to allow for proper directional airflow to maintain containment of all potential hazards and contaminants.

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REFERENCES

- NSF International (2018) NSF/ANSI 49 – 2018 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification. Ann Arbor, Michigan, USA.

- ISO (2015) ISO 14644-1:2015 Cleanrooms and associated controlled environments. Geneva, Switzerland.

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